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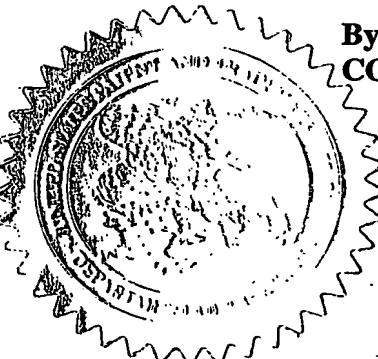
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## PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (280 characters max)		
PANCREATIC CANCER ASSOCIATED ANTIGEN, ANTIBODY THERETO, AND DIAGNOSTIC AND TREATMENT METHODS		
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CERTIFICATION UNDER 37 C.F.R. § 1.10  
I hereby certify that this correspondence and the documents referred to as enclosed are being deposited with the United States Postal Service on date below in an envelope as "Express Mail Post Office to Addressee" Mail Label Number EV253237503US addressed to: Box Provisional, Commissioner for Patents, Washington, D.C. 20231.

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**PANCREATIC CANCER ASSOCIATED ANTIGEN, ANTIBODY  
THERETO, AND DIAGNOSTIC AND TREATMENT METHODS**

**BACKGROUND OF THE INVENTION**

**1. Field of the Invention**

The present invention resides in the discovery of a specific antigen found on the  
10 surface of pancreatic carcinoma cells and monoclonal antibodies of high specificity and  
selectivity to the antigen. Both the antigen and antibodies thereto may be used in  
diagnosing and treating pancreatic cancer in an animal, especially a human.

**2. Description of the Related Art**

Pancreatic cancer is a nearly always fatal disease with a median survival time of  
15 only 80-90 days for a patient diagnosed with the disease. Pancreatic cancer is one of the  
more lethal forms of cancer in numbers of patients killed in the U.S. Less than 4% of  
patients are alive 5 years from the time of diagnosis, and none after approximately 7 years.  
These low survival rates are attributable to the fact that fewer than 10% of patients' tumors  
are confined to the pancreas at the time of diagnosis; in most cases, the malignancy has  
20 already progressed to the point where surgical removal has become impossible.  
Furthermore, in addition to the difficulty in accessing the pancreas and/or pancreatic tumor  
at its anatomical location, pancreatic cancer displays an unusual capacity of rapidly  
developing resistance against chemotherapy and radiotherapy. At present, no pancreatic  
cancer-specific markers, pancreatic cancer-specific antibodies, nor pancreatic cancer-  
25 specific assays exist that identify a pancreatic cancer-specific antigen in bodily fluids or

secretions.

One reason that pancreatic cancer (PaCa) claims 29,000 new lives every year in the U.S. alone and, therefore, occupies the fourth position in the cancer-related mortality hierarchy, is the lack of an early diagnostic tool. An effective early diagnostic tool  
5 requires a marker that is specific for PaCa and can be identified at a time when therapeutic intervention is successful in preventing progression of the lethal disease.

The present invention provides a pancreatic cancer associated antigen as well as antibodies which specifically bind to the antigen. The subject antigen and antibodies are useful in both methods of diagnosis and treatment of pancreatic cancer, also provided  
10 herein.

#### SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a pancreatic carcinoma-specific antigen 3C4-Ag in substantially purified form. 3C4-Ag may be characterized by a  
15 molecular weight of about 43 kDa as determined by SDS-PAGE; a pI on isoelectrofocusing of about 4.5 to about 5.0. 3C4-Ag is primarily localized on the surface of rat and human pancreatic cancer cells and is not detected in normal, non-proliferating cells. Immunologically active fragments of 3C4-Ag are also encompassed by the present invention.

20 Antibodies or binding portions thereof, having binding specificity to pancreatic carcinoma specific antigen 3C4-Ag are also provided wherein said antigen is characterized by a molecular weight of about 43 kDa as determined by SDS-PAGE; a pI on isoelectrofocusing of about 4.5 to about 5.0; and being primarily localized on the surface

of rat and human pancreatic cancer cells but not detected in normal, non-proliferating cells. Subject antibodies may be polyclonal or monoclonal and may also be in a humanized form. In addition, a subject antibody may be labeled with a fluorophore, chemilophore, chemiluminecer, photosensitizer, suspended particles, radioisotope or enzyme. In another 5 embodiment, a subject antibody may be conjugated or linked to a diagnostic, therapeutic drug, or toxin.

The present invention also provides Murine hybridoma cell lines which produce monoclonal antibodies specifically immunoreactive with the 3C4-Ag antigen.

In another aspect of the invention, there is provided a method of detecting 10 pancreatic cancer in an animal subject. The method comprises the steps of: (a) contacting a cell, tissue or fluid sample from the subject with at least one of an antibody or binding portion thereof which specifically binds to 3C4-Ag or an immunologically active fragment thereof; the monoclonal antibody mAb3C4; or an antibody which binds the epitope bound by the monoclonal antibody mAb3C4; under conditions permitting said antibody to 15 specifically bind an antigen in the sample to form an antibody-antigen complex; (b) detecting antibody-antigen complexes in the sample; and (c) correlating the detection of elevated levels of antibody-antigen complexes in the sample with the presence of pancreatic cancer.

In still another embodiment of the invention, there is provided a diagnostic kit 20 suitable for detecting 3C4-Ag in a cell, tissue, or fluid sample from a patient. The kit may comprise a number of different components such as: (a) an antibody or binding portion thereof which specifically binds 3C4-Ag or an immunologically active fragment thereof,

(b) a conjugate of a specific binding partner for the antibody or binding portion thereof; and (c) a label for detecting the bound antibody.

In another aspect of the invention, a method of treating pancreatic cancer in a patient is provided. The method comprises the steps of administering to the patient an effective amount of an antibody or binding portion thereof which specifically binds to 3C4-Ag or an immunologically active fragment thereof, wherein said antibody or binding portion thereof is conjugated or linked to a therapeutic drug or toxin.

A pharmaceutical composition comprising an antibody or binding portion thereof which specifically binds to 3C4-Ag, admixed with a pharmaceutically acceptable carrier is also provided. The antibody or binding portion thereof which specifically binds to 3C4-Ag may be conjugated or linked to a therapeutic drug or toxin in the pharmaceutical composition.

#### BRIEF DESCRIPTION OF THE FIGURES

Figures 1A through 1F are photomicrographs showing morphological changes induced by NNK in BMRPA1 cells. Figure 1A shows normal appearance of untreated BMRPA1 cells. Figures 1B through 1F show sequential cell passages (1-12) after one 16h treatment of BMRPA1 with NNK.

Figures 2A through 2C are photomicrographs of immunofluorescence (IF) stained live BMRPA1.NNK cells with ISHIP mice serum (A), with 3C4 hybridoma spent medium (B) and BMRPA1 cells with 3C4 hybridoma spent medium (C). The surface expression of the 3C4-Ag on BMRPA1.NNK cells is clearly apparent in FIGURE 2B in the linear ring-like fluorescence image while the BMRPA1 cells are completely devoid of any staining.

Figure 3, lanes 1-4, is a photograph of a stained SDS gel run with G-protein affinity purified mAb3C4 from ascites. Lane 1: hybridoma injected mouse ascites; Lane 2: low pH elution where IgG was quantitatively released from the bead. Lane 3 shows the ~160 kD protein (IgG) of lane 3 reduced. Lanes 1B and 2B depict immunoblots and autoradiograms (chemiluminescentograms) of the IgG in lanes 1 and 2 using HRP-SaM IgG and ECL reaction kit, confirming the ~160 kD protein to be IgG.

Figure 4 is an autoradiograph showing SDS PAGE of cell lysate proteins from rodent and human pancreatic carcinoma cells, followed by an immunoblot with mAb3C4.

Figure 5A is gel photograph showing silver stained lysates of BMRPA1.NNK cells processed without mAb3C4 (lane 1) and with mAb3C4 and protein G beads (lane 2). Figure 5B is an immunoblot for the 3C4-Ag in the immunoprecipitates from the lysates in Figure 5A (BMRPA1.NNK cells). Immunoprecipitate obtained (lane 1) without mAb3C4, IB with mAb3C4 and HRP-SaM IgG; (lane 2) with mAb3C4, IB with mAb3C4 and HRP-SaM IgG identifying the 3C4-Ag as 43kD polypeptide; (lane 3) with mAb3C4, IB without mAb3C4 but with HRP-SaM IgG.

Figures 6A, 6C, 6E, 6G, and 6I are phase contrast visible light photomicrographs of live rodent and human pancreas carcinoma cells stained with mAb3C4. Figures 6B, 6D, 6F, 6H, and 6J are UV light photographs processed identically and showing membrane fluorescence. Figures 6A and 6B: BMRPA1.NNK cells; Figures 6C and 6D: BMRPA1.TUC3 cells; Figures 6E and 6F: CAPAN-1 cells; Figures 6G and 6H: CAPA2-2 cells; 6I and 6J are BxPC3 cells. 6A -6D are rodent pancreatic carcinoma cells. 6E-6J are human pancreatic carcinoma cells.

Figure 7 shows Fluorescent Activated Cell Sorting (FACS) analysis of transformed and untransformed rodent and human PaCa cells. (A) BMRPA1.Tuc3; (B) BMRPA1.NNK; (C) human MIA PaCa. Left panels are scattergrams identifying the cell population examined for binding of mAb3C4. Right panels show fluorescence intensity of the selected cell population. Peaks labeled (1) indicate background fluorescence by processing the cells with FITC-R- $\alpha$ M IgG only (no primary antibody)(background control); (2) cells reacted with mAb3C4 and FITC-R- $\alpha$ M IgG.

Figure 8 graphically depicts cytotoxicity of mAb3C4. X axis: rabbit serum (complement) dilutions; Y axis: percentage of cells alive after exposure to mAb3C4 and rabbit complement. The first bar of each group shows treatment of cells with fresh rabbit serum only (source of complement) for 45 minutes at 37° C. The second bar of each group represents cells treated with mAb3C4 and rabbit serum (source of complement) for 45 minutes at 37 °C. The third bar of the first group represents cells treated with mAb3C4 followed by heat inactivated (30-45 minutes at 56° C) rabbit serum (inactivated complement).

Figures 9A and 9B are immunoblots of tissue extracts using mAb3C4; Figure 9A:rat; Figure 9B:human. Reduced proteins from tissue extracts from various tissues (thyroid, ovary, brain, heart, lung, liver, testes, Fig. 9A) as well as human acinar pancreatic cells, white blood cells, and ductal pancreatic cells were separated on 12% SDS PAGE, electrophoretically transferred to nitrocellulose and processed with and without mAb3C4 followed by ECL chemiluminescence amplification. MIA-PaCa and mouse IgG served as controls. "+" means reaction with primary mAb. "-" means no reaction with primary

mAb. MIA-PaCa and mouse IgG served as negative controls.

"\*" indicates tissue extract was obtained by Dounze homogenization in the presence of Triton X-100 containing lysing buffer. "#" indicates tissue extract was obtained by high frequency pulse sonication in the presence of Triton X-100 containing lysing buffer.

5       Figure 10 show autoradiographs of immunoblots of various cancerous tissues using mAb3C4.

Figure 11 is a gel photo of proteins of BMRPA1.NNK cell lysates separated by two dimensional gel (2-D-Gel) electrophoresis according to size and pI, and identified by silver staining.

10      Figure 12 is a chemiluminescentogram showing the proteins of BMRPA1.NNK cell lysates separated by 2D-Gel-electrophoresis as described for Figure 11, electrophoretically transferred to PVDF membrane and blotted with mAb3C4.

Figure 13 graphically depicts the effect of *in vivo* administration of mAb3C4 on tumor growth.

15

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a pancreatic carcinoma-specific antigen and antibodies which specifically bind thereto. The pancreatic carcinoma-specific antigen (pancreatic cancer associated antigen), also referred to hereinafter as 3C4-Ag, has a  
20 molecular weight of about 43 kDa as determined by SDS polyacrylamide electrophoresis (SDS PAGE) and is primarily localized on the surface of pancreatic cancer cells. 3C4-Ag is not detected in normal, non-proliferating cells and is only detected at very low levels in

renal, prostate and possibly colon carcinoma.

3C4-Ag was initially identified by indirect immuno-fluorescence (IF) on intact, live and intact, fixed pancreatic cancer cells (rat and human cell lines) as a cell surface antigen, using a mouse monoclonal antibody, mAbC4, as a primary antibody, followed by 5 fluorescein-labeled sheep or rabbit anti-mouse IgG (FITC-S or R anti-M IgG) and fluorescence microscopy. The monoclonal antibody mAb3C4 was produced using an immunosubtractive-hyperimmunization protocol (ISHIP), which protocol is fully described in Applicants' Provisional Patent Application, entitled "Tolerance-Induced Targeted Antibody Production (TITAP)," filed \_\_\_\_\_, 2002, Attorney Docket 1181-9, 10 U.S. Serial Number 60/\_\_\_\_\_, the disclosure of which is incorporated by reference herein as if fully set forth. In accordance with the ISHIP protocol, cyclophosphamide-induced tolerance in a mouse to antigens present on untransformed rat pancreatic cells (BMRP1 cells) followed by subsequent hyper-immunizations with BMRPA1 cells neoplastically transformed with the known carcinogen 4-(methyl-nitrosamino)-1-(3- 15 pyridyl)-1-butanone (hereinafter BMRP1.NNK cells), resulted in increased immigration of plasma cells secreting antibodies to BMRPA1.NNK cells into the spleen of the mouse. Subsequent fusion of splenocytes from immunized mice with P3U1 myeloma cells resulted in the production of hybridomas secreting antibodies which specifically react with a 20 pancreatic cancer associated antigen (3C4-Ag) on the surface of BMRPA1.NNK , but not untransformed cells.

In accordance with the present invention, there is provided a pancreatic carcinoma specific antigen 3C4-Ag in substantially purified form. The 3C4-Ag is characterized by:

a molecular weight of about 43 kDa as determined by SDS-PAGE; a pI on  
isoelectrofocusing of about 4.5 to about 5.0; and being soluble in 50mM Tris-HCl, 1%  
NP40, 0.5% sodium deoxycholate, 0.1% SDS, 5mM EDTA, 1 µg/mL pepstatin, 2 ug/mL  
aprotinin, 1 mM PMSF, and 5mM iodoacetamide, and; being primarily localized on the  
5 surface of rat and human pancreatic cancer cells but not detected in normal, non-  
proliferating cells.

Also in accordance with the present invention, there is provided an antibody having  
binding specificity to pancreatic carcinoma specific antigen 3C4-Ag, wherein said antigen  
is characterized by a molecular weight of about 43 kDa as determined by SDS-PAGE;  
10 a pI on isoelectrofocusing of about 4.5 to about 5.0; and being soluble in 50mM Tris-HCl,  
1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 5mM EDTA, 1 µg/mL pepstatin, 2  
ug/mL aprotinin, 1 mM PMSF, and 5mM iodoacetamide, and; being primarily localized on  
the surface of rat and human pancreatic cancer cells but not detected in normal, non-  
proliferating cells. A subject antibody which specifically binds to 3C4-Ag may be a  
15 polyclonal or monoclonal antibody. Preferably, the antibody is a monoclonal antibody  
(mAb). Even more preferably, the mAb is 3C4.

A murine hybridoma cell line which produces a monoclonal antibody specifically  
immunoreactive with 3C4-Ag is also provided. Preferably, the murine hybridoma cell line  
produces mAb3C4.

20 The pancreatic cancer associated antigen 3C4-Ag, may be prepared using a number  
of well known methods. 3C4-Ag may be identified and its gene sequence obtained using  
an immunosubtractive hybridization or differential RNA display methodology. A gene

encoding the 3C4-Ag under control of a promoter which functions in a particular host cell may be used to transfect such a host cell in order to express the antigen. Alternatively, 3C4-Ag may be chemically synthesized using well known methods.

Pancreatic cancer associated antigen 3C4-Ag may be purified using well known  
5 methods in the art such as polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol., 182:488-495), and size-exclusion chromatography. Other purification techniques, such as immunoaffinity chromatography using an antibody which binds 3C4-Ag such as mAb3C4, may also be performed. Such methods are exemplified herein in Example 8. Following SDS PAGE, the 3C4-Ag band of  
10 about 43 kDa may be excised from the gel and eluted into an appropriate buffer. Further purification of 3C4-Ag may be performed including gel filtration, ion exchange chromatography and/or high performance liquid chromatography (HPLC). HPLC is the preferred method of purification.

Purified 3C4-Ag or an immunologically fragment thereof, may be used to inoculate  
15 an animal in order to produce polyclonal antibodies which react with 3C4-Ag. By "immunologically active fragment" is meant a fragment of the approximately 43kDa 3C4-Ag protein which fragment is sufficient to stimulate production of antibodies which specifically react with an exposed epitope on 3C4-Ag as 3C4-Ag is exposed on the surface of pancreatic cancer cells. Thus, in addition to mAb3C4, the present invention  
20 contemplates other antibodies, polyclonal or monoclonal, which specifically react with 3C4-Ag or an immunologically active fragment thereof and which antibodies may or may not bind to the same epitope on 3C4-Ag as does mAb3C4.

Animals, for example, mammals such as mice, goats, rats, sheep or rabbits, or other animals such as poultry, e.g., chickens, can be inoculated with 3C4-Ag or immunologically active fragment thereof, preferably conjugated with a suitable carrier protein to produce polyclonal antibodies. Such immunizations may be repeated as necessary at intervals of up to several weeks in order to obtain a sufficient titer of antibodies. Blood is collected from the animal to determine if antibodies are produced, the antisera is tested for response to the 3C4-Ag or immunologically active fragment thereof, and reboosting is undertaken, as needed. In some instances, after the last antigen boost, the animal is sacrificed and spleen cells removed. Immunoglobulins are purified from the serum obtained from the 10 immunized animals. These immunoglobulins can then be utilized in diagnostic immunoassays to detect the presence of antigen in a sample, or in therapeutic applications.

Preferably, monoclonal antibodies which specifically react against 3C4-Ag or immunologically active fragment thereof are prepared. Methods of producing monoclonal antibodies are well known in the art such as described in Kohler and Milstein (1975) 15 *Nature* 256:495-497, which is incorporated by reference herein as if fully set forth. For example, an animal may be immunized with 3C4-Ag or immunologically active fragment thereof, and spleen cells from the immunized animal obtained. The antibody-secreting lymphocytes are then fused with myeloma cells or transformed cells which are capable of replicating indefinitely in cell culture. Resulting hybridomas may be cultured and the 20 resulting colonies screened for the production of the desired monoclonal antibodies. Antibody producing colonies may be grown either *in vivo* or *in vitro* in order to produce large amounts of antibody.

The hybridoma cell line may be propagated *in vitro*, and the culture medium containing high concentrations of the mAb (such as mAb3C4) harvested by decantation, filtration, or centrifugation. Alternatively, a sample of a subject antibody such as mAb3C4 may be injected into a histocompatible animal of the type used to provide the somatic and 5 myeloma cells for the original fusion, e.g., a mouse. Tumors secreting the mAb develop in the injected animal and body fluids of the animal; such as ascites, fluid, or serum produce mAb in high concentrations.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, 10 for example, by using polyethylene glycol (PEG) or other fusing agents such as described in Milstein and Kohler (1976) *Eur. J. Immunol.* 6:511, Brown et al. (1981) *J. Immunol.* 127(2):539-46, Brown et al. (1980) *J. Biol. Chem.*, 255:4980-83, and Yeh et al., *Proc. Nat'l. Acad. Sci. (USA)* 76(6):2927-31, which disclosures are incorporated by reference herein as if fully set forth. Such an immortal cell line is preferably murine, but may also be derived 15 from cells of other mammalian species such as rats and human. Preferably, the cell line is deficient in enzymes necessary for the utilization of certain nutrients, is capable of rapid growth and has a good fusion capability. Such cell lines are known to those skilled in the art.

Methods for purifying monoclonal antibodies include ammonium sulfate 20 precipitation, ion exchange chromatography, and affinity chromatography such as described in Zola et al. in *Monoclonal Hybridoma Antibodies: Techniques and Applications*, Hurell (ed)pp. 5-52 (CRC Press 1982) the disclosure of which is

incorporated by reference herein as if fully set forth. As described in the present application, Example 7, mice may be injected with 3C4 hybridoma cells, followed by collection of ascites. mAb3C4 may be purified from the ascites using G-protein affinity beads. After washing the beads in an appropriate buffer, the bound mAb3C4 may be 5 eluted from the beads with an elution buffer and separated by the beads by brief centrifugation.

In addition to utilizing whole antibodies, the methods of the present invention encompass use of binding portions of antibodies which specifically bind 3C4-Ag or an immunologically active fragment thereof. Such binding portions include Fab fragments, 10 F(ab')2 fragments, and Fc fragments. These antibody fragments may be made by conventional procedures, such as proteolytic fragmentation procedures, as described in Goding, *Monoclonal Antibodies:Principles and Practice*, pp. 98-118, New York, Academic Press (1983), which is incorporated by reference herein as if fully set forth.

The present invention also provides diagnostic methods for detecting pancreatic 15 cancer in a patient. The diagnostic methods are based on immunoassays which detect the presence of pancreatic carcinoma specific antigen (3C4-Ag) in a sample from a patient by reacting with a subject antibody which specifically binds 3C4-Ag or an immunologically active fragment thereof. Examples of patient sample sources include cells, tissue, tissue lysate, tissue extract, or blood-derived sample (such as blood, serum, or plasma), urine, or 20 feces. Preferably, the sample is fluid. The fluid sample is preferably blood serum but could be other fluids such as pleural or ascitic fluid. A detected increase in the level of 3C4-Ag in a sample correlates with a diagnosis of pancreatic cancer in the patient.

There are many different types of immunoassays which may be used in the methods of the present invention. Any of the well known immunoassays may be adapted to detect the level of 3C4-Ag in a serum sample or other sample of a patient, which reacts with an antibody which specifically binds 3C4-Ag, such as, e.g., enzyme linked immunoabsorbent assay (ELISA), fluorescent immunosorbent assay (FIA), chemical linked immunosorbent assay (CLIA), radioimmuno assay (RIA), and immunoblotting. For a review of the different immunoassays which may be used, see: *The Immunoassay Handbook*, David Wild, ed., Stockton Press, New York, 1994; Sikora et al. (eds.), *Monoclonal Antibodies*, pp. 32-52, Blackwell Scientific Publications (1984).

For example, an immunoassay to detect pancreatic cancer in a patient involves contacting a sample from a patient with a first antibody or binding portion thereof (e.g., mAb3C4), which is preferably soluble and detectable to form an antibody-antigen complex with 3C4-Ag in the sample. The complex is contacted with a second antibody which recognizes constant regions of the heavy chains of the first antibody. For example, the second antibody may be an antibody which recognizes constant regions of the heavy chains of mouse immunoglobulin which has reacted with mAb3C4 (anti-mouse antibody). The second antibody is labeled with a fluorophore, chemilophore, chemiluminescer, photosensitizer, suspended particles, or radioisotope. Free labeled second antibody is separated from bound antibody. The signal generated by the sample is then measured depending on the signal producing system used. Increased optical density or radioactivity when compared to samples from normal patients correlates with a diagnosis of pancreatic cancer in a patient.

Alternatively, an enzyme-labeled antibody such as e.g.,  $\beta$ -galactosidase-labeled antibody, is used and an appropriate substrate with which the enzyme label reacts is added and allowed to incubate. Enzymes may be covalently linked to 3C4-Ag reactive antibodies for use in the methods of the invention using well known conjugation methods. For example, alkaline phosphatase and horseradish peroxidase may be conjugated to antibodies using glutaraldehyde. Horseradish peroxidase may also be conjugated using the periodate method. Commercial kits for enzyme conjugating antibodies are widely available. Enzyme conjugated anti-human and anti-mouse immunoglobulin specific antibodies are available from multiple commercial sources.

Enzyme labeled antibodies produce different signal sources, depending on the substrate. Signal generation involves the addition of substrate to the reaction mixture. Common peroxidase substrates include ABTS® (2,2'-azinobis(ethylbenzothiazoline-6-sulfonate)), OPD (O-phenylenediamine) and TMB (3,3', 5,5'-tetramethylbenzidine). These substrates require the presence of hydrogen peroxide. *p*-nitrophenyl phosphate is a commonly used alkaline phosphatase substrate. During an incubation period, the enzyme gradually converts a proportion of the substrate to its end product. At the end of the incubation period, a stopping reagent is added which stops enzyme activity. Signal strength is determined by measuring optical density, usually via spectrophotometer.

Alkaline phosphatase labeled antibodies may also be measured by fluorometry. Thus in the immunoassays of the present invention, the substrate 4-methylumbelliferyl phosphate (4-UMP) may be used. Alkaline phosphatase dephosphorylates 4-UMP to form 4-methylumbelliferone (4-MU), the fluorophore. Incident light is at 365 nm and emitted

light is at 448 nm..

As an alternative to enzyme-labeled antibodies, fluorescent compounds, such as fluorescein, rhodamine, phycoerytherin, indocyanine, biotin, phycocyanine, cyanine 5, cyanine 5.5, cyanine 7, cyanine 3, aminomethyl cumarin (AMCA), peridinin chlorophyl,  
5 Spectral red, or Texas red may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labeled antibody is allowed to bind  
10 to the first antibody-hapten complex. After washing off the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength. The fluorescence observed indicates the presence of the hapten of interest, in this case 3C4-Ag. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as  
15 radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purposes.

A subject antibody may also be detected with a group of secondary labeled ligands which are capable of binding to the antibody. For example, using conventional techniques  
20 biotin may be linked to antibodies produced according to the present invention. The biotinylated antibody is then allowed to contact and bind 3C4-Ag. Streptavidin or avidin which has been labeled with a known label is then contacted with the antibody/3C4-Ag

complex which then leads to binding of the labeled streptavidin or avidin to the biotin portion of the biotinylated antibody. Additional biotin may be added followed by the addition of more labeled streptavidin or avidin. Since each streptavidin or avidin molecule is capable of binding four biotin molecules, a relatively large three-dimensional network is  
5 created which includes numerous labels which may be detected by conventional fluorescence microscopy or by radiographic techniques.

Other immunoassay techniques are available for utilization in the present invention as shown by reference to U.S. Pat. Nos. 4,016,043; 4,424,279; and 4,018,653. This, of course, includes both single-site and two-site, or "sandwich", assays of the non-competitive  
10 types, as well as the traditional competitive binding assays described above. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention.

In the typical forward sandwich assay, a first antibody having specificity for 3C4-Ag or an immunologically active fragment thereof, is either covalently or passively bound  
15 to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking, covalently  
20 binding, or physically adsorbing the molecule to the insoluble carrier. Following binding, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period

of time sufficient to allow binding to the antibody. The incubation period will vary, but will generally be in the range of about 2-40 minutes. Following the incubation period, the antibody subunit-solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule 5 which is used to indicate the binding of the second antibody to the hapten.

Variations on the forward assay include a simultaneous assay, in which both sample and labeled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labeled antibody and sample to be tested are first combined, incubated and then added to the unlabeled surface bound antibody. These techniques are well known 10 to those skilled in the art, and the possibility of minor variations will be readily apparent to those skilled in the art.

Cross-linkers suitable for use in coupling a label to an antibody are well-known. Homofunctional and heterobifunctional cross-linkers are all suitable. Reactive groups which can be cross-linked with a cross-linker include primary amines, sulphydryls, 15 carbonyls, carbohydrates and carboxylic acids. Cross-linkers are available with varying lengths of spacer arms or bridges. Cross-linkers suitable for reacting with primary amines include homobifunctional cross-linkers such as imidoesters and N-hydroxysuccinimidyl (NHS) esters.

Heterobifunctional cross-linkers which possess two or more different reactive 20 groups are suitable for use herein. Examples include cross-linkers which are amine-reactive at one end and sulphydryl-reactive at the other end such as 4-succinimidyl-

oxycarbonyl- $\alpha$ -(2-pyridylidithio)-toluene, N-succinimidyl-3-(2-pyridylidithio)-propionate and maleimide cross-linkers.

The amount of color, fluorescence, luminescence, or radioactivity present in the reaction (depending on the signal producing system used) is proportionate to the amount of

- 5 3C4-Ag in a patient's sample which reacts with a subject antibody such as mAb3C4.

Quantification of optical density may be performed using spectrophotometric methods.

Quantification of radiolabel signal may be performed using scintillation counting.

Increased levels of 3C4-Ag reacting with a subject antibody such mAb3C4 over normal sample levels correlate with a diagnosis of pancreatic cancer in the patient.

- 10 The present invention also provides diagnostic kits for performing the methods described hereinabove. In one embodiment, the diagnostic kit comprises: (i) an antibody or binding portion thereof, which specifically binds to 3C4-Ag or an immunologically active fragment thereof, (ii) a conjugate of a specific binding partner for the antibody, and (iii) a label for detecting the bound antibody. In a preferred embodiment, the antibody  
15 which specifically binds to 3C4-Ag is mAb3C4. An example of a conjugate of a specific binding partner for mAb3C4 is an antibody which specifically binds to mAb3C4. If the label is an enzyme, then a third container, containing a substrate for the enzyme may be provided.

- The kit may also comprise other components such as buffering agents and protein  
20 stabilizing agents, e.g., polysaccharides, and the like. In addition, a subject kit may comprise other agents of the signal-producing system such as agents for reducing background interference, control reagents, and compositions suitable for conducting the

diagnostic test. Such compositions may include for example, solid surfaces such as glass or polymer such as cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. Solid supports may be in the form of tubes, beads, discs, or microplates, or any other surface for conducting an immunoassay.

5       The antibodies of the present invention are also useful for *in vivo* diagnostic applications for the detection of pancreatic tumors, preferably human. For example, pancreatic tumors may be detected by tumor imaging techniques using mAb34C labeled with an appropriate imaging reagent that produces detectable signal. Imaging reagents and procedures for labeling antibodies with such reagents are well known. *See e.g.*, Wensel and Meares, *Radio Immunoimaging and Radioimmunotherapy*, Esevier, New York (1983);  
10      Colcher et al., *Meth. Enzymol.* 121:802-816 (1986). The labeled antibody may then be detected by e.g., radionuclear scanning as described in Bradwell et al. *Monoclonal Antibodies for Cancer Detection and Therapy*, Baldwin et al. (eds), pp. 65-85, Academic Press (1985).

15       In accordance with the present invention, there are also provided therapeutic methods for treating a patient suffering from pancreatic cancer. For example, the mAb3C4 may be used alone to target tumor cells or used in conjunction with an appropriate therapeutic agent to treat pancreatic cancer. When a subject antibody which binds 3C4-Ag or an immunologically active fragment thereof, is used alone, such treatment can be  
20      effected by initiating endogenous host immune functions, such as complement-mediated or antibody-dependent cellular cytotoxicity (ADCC). ADCC involves an antibody which can kill cancer cells in the presence of human lymphocytes or macrohages or becomes

cytotoxic to tumor cells in the presence of human complement. An antibody of the present invention, which specifically reacts with 3C4-Ag may be modified for ADCC using techniques developed for the production of chimeric antibodies as described by Oi et.al., (1986) *Biotechnologies* 4(3):214-221; and Fell et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:8507-8511.

In a preferred embodiment, a subject antibody which specifically binds 3C4-Ag or an immunologically active fragment thereof, may be conjugated or linked to a therapeutic drug or toxin for delivery of the therapeutic agent to the site of cancer. Enzymatically active toxins and fragments thereof include but are not limited to: diphtheria toxin A fragment, nonbonding active fragments of diphtheria toxin, exotoxin A from *Pseudomonas aeruginosa*, ricin A chain, abrin A chain, modeccin A chain,  $\alpha$ -sacrin, certain *Aleurites fordii* proteins, certain Dianthin proteins, *Phytolacca americana* proteins (PAP, PAPII and PAP-S), *Morodica charantia* inhibitor, curcin, crotin, *Saponaria officinalis* inhibitor, gelonin, mitogillin, restrictocin, phenomycin, enomycin, and derivatives (including synthetic) of taxol, for example. International Patent Publications WO 84/03508 and WO 85/03508, incorporated by reference herein as if fully set forth, describe procedures for preparing enzymatically active polypeptides of such immunotoxins.

Other cytotoxic moieties include but are not limited to those derived from adriamycin, chlorambucil, daunomycin, methotrexate, neocarzinostatin, and platinum. Procedures for conjugating chlorambucil with antibodies are described in Flechner (1973) *European J. Cancer* 9:741-745; Ghose et al. (1972) *British Medical J.* 3:495-499, and Szekerke et al., (1972) *Neoplasma* 19:211-215, which are incorporated by reference herein

as if fully set forth. Procedures for conjugating daunomycin and adriamycin to antibodies are described in Hurwitz et al. (1975) *Cancer Research* 35:1175-1181 and Arnon et al., (1982) *Cancer Surveys* 1:429-449, the disclosures of which are also incorporated by reference herein as if fully set forth. Procedures for preparing antibody-ricin conjugates 5 are described e.g., in U.S. Patent No. 4,414,148 and in Osawa et al., (1982) *Cancer Surveys* 1:373-388 as well as the references cited therein, which are incorporated by reference herein as if fully set forth. European Patent Application 86309516.2 also describes coupling procedures and is incorporated by reference herein.

Antibodies to 3C4-Ag and binding portions thereof may also be used in a 10 drug/prodrug treatment regimen. For example, a first antibody or binding portion thereof according to the present invention is conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second antibody or binding portion thereof, preferably one which binds to pancreatic cancer cells or to other biological materials associated with pancreatic cancer 15 cells such as another protein produced by the diseased pancreas cells. See e.g., Senter et al. (1988) *Proc. Nat'l. Acad. Sci. (USA)* 85:4842-46; and Blakely et al., (1996) *Cancer Res.* 56:3287-3292, both of which are incorporated by reference as if fully set forth.

Alternatively, the antibody or binding portion thereof may be coupled to a high 20 energy radiation emitter, e.g., a radioisotope such as  $^{131}\text{I}$ , a  $\gamma$  emitter, which when localized at a tumor site, results in a killing of several cell diameters. See e.g., Order, in *Monoclonal Antibodies for Cancer Detection and Therapy*, Baldwin et al. (eds.) pp.303-16, Academic Press, (1985).  $^{67}\text{Cu}$  is also effective and may be attached to a subject antibody via an

appropriate metal chelator which is bound to the antibody. Other suitable radioisotopes include  $\alpha$ -emitters such as  $^{212}\text{Bi}$ ,  $^{213}\text{Bi}$ , and  $^{211}\text{At}$  and  $\beta$ -emitters, such as  $^{186}\text{Re}$  and  $^{90}\text{Y}$ .

For therapeutic applications, chimeric (mouse-human) humanized monoclonal antibodies may be preferable to murine antibodies, since human subjects treated with mouse antibodies tend to generate antimouse antibodies. Antibodies may be "humanized" by designing and synthesizing composite variable regions which contain the amino acids of the mouse complementary determining regions (CDRs) integrated into the framework regions (FRs) of a human antibody variable region. Resultant antibodies retain the specificity and binding affinity of the original mouse antibody but are sufficiently human so that a patient's immune system will not recognize such antibodies as foreign.

Techniques for humanizing mouse monoclonal antibodies include for example, those described in Vaswani et al., (1998) *Ann. Allergy Asthma Immunol.* 81:105-119 and U.S. Patent No. 5,766,886 to Studnicka et al., the disclosures of which are incorporated by reference herein as if fully set forth.

The present invention further provides pharmaceutical compositions which may be used in the therapeutic methods described hereinabove. The pharmaceutical compositions comprise a pharmaceutically effective amount of an antibody or binding portion thereof which specifically recognizes and binds to 3C4-Ag or an immunologically active fragment thereof, and a pharmaceutically acceptable carrier. Examples of pharmaceutically acceptable carriers include sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable carrier, including adjuvants, excipients, or stabilizers. Illustrative oils are those of petroleum,

animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols, such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Human serum albumin, ion exchangers; alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate may also be used.

5 A subject pharmaceutical composition therefore comprises an antibody or binding portion thereof which specifically binds to 3C4-Ag or immunologically active fragment thereof, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme, or second antibody as described hereinabove) or in a recombinant form such as a chimeric 10 Ab. The pharmaceutical composition may additionally comprise other antibodies or conjugates for treating pancreatic cancer, such as e.g., an antibody cocktail.

Regardless of whether the antibodies or binding portions thereof of the present invention are used for treatment or *in vivo* detection of pancreatic cancer, they can be 15 administered orally, parenterally, subcutaneously, intravenously, intralymphatic intramuscularly, intraperitoneally, by intranasal instillation, by intracavitory or intravesical instillation, intraarterially, intralesionally, or applied to tissue surfaces (including tumor surfaces or directly into a tumor) in the course of surgery. The antibodies of the present invention may be administered alone or with pharmaceutically or physiologically 20 acceptable carriers, excipients, or stabilizers as described hereinabove. The subject antibodies may be in solid or liquid form such as tablets, capsules, powders, solutions,

suspensions, emulsions, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions.

Effective modes of administration and dosage regimen for the antibody compositions of the present invention depend mostly upon the patient's age, weight, and progression of the disease. Dosages should therefore be tailored to the individual patient. Generally speaking, an effective does of the antibody compositions of the present invention may be in the range of from about 1 to about 5000 mg/m<sup>2</sup>.

The following examples further illustrate the invention and are not meant to limit the scope thereof.

10

**EXAMPLE 1****Preparation of cell lines**

Materials: 1640 RPMI medium, penicillin-streptomycin stock solution

5 (10,000U/10,000mg/mL)(P/S), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer, 0.2% Trypsin with 2mM Ethylene diamine tetraacetic acid (Trypsin-EDTA), and Trypan blue were all from GIBCO (New York). Fetal bovine serum (FBS) was from Atlanta Biologicals (Atlanta, GA). Dulbecco's Phosphate Buffered Saline without Ca<sup>2+</sup> and Mg<sup>2+</sup> (PBS), and all trace elements for the complete medium were  
10 purchased from Sigma Chemical Company (ST. Louis, MO). Tissue culture flasks (TCFs) were from Falcon- Becton Dickinson (Mountain View, C.A.), tissue culture dishes (TCDs) were obtained from Corning (Corning, NY), 24-well tissue culture plates (TCP), and 96-well TCP were from Costar (Cambridge, MA). Filters (0.22, 0.45μm) were from Nalgene (Rochester, NY).

15 Preparation of complex RPMI (cRPMI) cell culture medium: cRPMI was prepared with RPMI, glutamine (0.02M), HEPES-Buffer (0.3M), bovine insulin dissolved in acetic acid (0.02 mg/mL acetic acid/L of medium), hydrocortisone (0.1μg/mL), trace elements that included ZnSO<sub>4</sub> (5X10<sup>-7</sup>M), NiSO<sub>4</sub> 6H<sub>2</sub>O (5X10<sup>-10</sup> M), CuSO<sub>4</sub> (10<sup>-8</sup>M), FeSO<sub>4</sub> (10<sup>-6</sup>M), MnSO<sub>4</sub> (10<sup>-9</sup>M), (NH<sub>4</sub>)<sub>6</sub>Mn<sub>7</sub>O<sub>24</sub> (10<sup>-7</sup>M), Na<sub>2</sub>SeO<sub>3</sub> (0.5mg/L medium), SnCl<sub>2</sub> 20 2H<sub>2</sub>O(5X10<sup>-10</sup>M) and carbamyl choline (10<sup>-5</sup>M), and the pH was adjusted to 7.3. The medium was steriley filtered.

Cells and Culture: BMRPA1 cells are normal rat pancreatic acinar cells maintained in culture. These cells exhibit the differentiated characteristics of normal

epithelial cells and the biosynthetic secretory responses of pancreatic acinar cells (Bao et al., 1996). BMRPA1.TUC-3 (also known as TUC-3 or BMRPA.K-ras Val<sup>12</sup>) are BMRPA1 cells transformed *in vitro* by the direct transfection of a human activated p21 k-ras minigene containing a point mutation (GGT to GTT) in codon 12 (k-rasval12)(Dr. M. 5 Perucho, California Institute for Biological Research, La Jolla). TUC-3 cells have lost the epithelial phenotype and instead display *in vitro* and *in vivo* (tumor and metastasis formation) the characteristics of pancreatic carcinoma cells. MIA PaCa-2 (CRL1420), BxPC-3, CAPAN-1 and CAPAN-2 are human pancreatic carcinoma cells (Yunis et al., 1977) purchased from ATCC laboratories and grown in DMEM with 15% FBS.

10 BMRPA.430 (BMRPA1) is a spontaneously immortalized cell line established from normal rat pancreas (Bao et al, 1994). The BMRA1 cell line is maintained routinely in cMEM (10% FBS). All cell lines are grown in a 95% air-5% CO<sub>2</sub> incubator (Forma Scientific) at 37°C and passaged by trypsin-EDTA. Cells are stored frozen in a mixture made of 50% spent medium and 50% freezing medium containing fresh cMEM with 10% 15 FBS and 10% DMSO. Cell viability is routinely assessed by trypan blue exclusion.

NNK Exposures: All preparations of the carcinogen-containing media were made in a separate laboratory within a NCI-designed and certified chemical hood using prescribed protective measures. NNK (American Health Foundation, N.Y.) was prepared as a stock solution of 10mg NNK in PBS and added to FBS-free cMEM to make final 20 concentrations of 100, 50, 10, 5, and 1µg/ml. BMRPA1 cells at passage 36 (p36) were seeded at 10<sup>5</sup>/60mm TCDs and allowed to grow for 6 d. At this time the medium was removed, and the cells were washed 2x with prewarmed (37°C), FBS-free cMEM before

they were treated with FBS-free cMEM (4ml/TCD) containing the different concentrations of NNK. A 6th set of TCDs containing BMRPA1 cells was incubated in FBS-free cMEM without NNK and was used as controls. The eight TCDs used for each of the six sets of different culture conditions were returned to the 37°C and 95% air-5% CO<sub>2</sub> incubator.

5 After 16h, the NNK-containing medium was removed from all TCDs and the cells were washed 3x with PBS followed by addition of fresh cMEM-10% FBS (4ml/TCD), and the incubation continued. Control cultures without NNK were processed in parallel. The cells were fed every 2d by replacing 1/2 of the spent medium with fresh cMEM-10% FBS. At full confluence the cells were collected from all TCDs, the cells in each group were

10 pooled, and passaged at 2X10<sup>4</sup> into fresh TCDs. From each passage, cells not used for immediate culture or other procedures were frozen in liquid nitrogen for future analysis.

Isolation of Colonies: To facilitate the picking of cells from individual colonies of transformed cells, cell cultures containing colonies were reseeded at 10<sup>5</sup> cells/100mm TCDs, and grown for 7 d. The narrow ends of sterile Pasteur pipettes were flamed, rapidly stretched and broken at their thinnest point to create a finely drawn-out glass needle narrow enough to pick up only the core of a cell-rich colony. Only the NNK-treated cells contained cell-rich, ball-like colonies. The center cores of 8 prominent colonies were picked, and each core consisting of ~80-200 tightly packed cells was placed into a separate well each of a 24-well dish. The cells of 4 colonies thus transferred survived and were

15 expanded.

Further characterization of NNK-treated cells, including selection of BMPRA1 cells exposed to a single treatment (16 hours) of 1 ug NNK/mL FBS-free medium

(hereinafter termed BMRPA1.NNK cells) and evidence for the neoplastic nature of BMRPA1.NNK cells based on *inter alia*, observed ability of such cells to sustain cell growth in severely-deprived conditions, to grow on agar under anchorage independent conditions, and to grow as tumors in Nu/Nu mice, are described in copending provisional 5 patent application, entitled "Tolerance-Induced Targeted Antibody Production (TTIAP)," filed \_\_\_\_\_, 2002, Attorney Docket 1181-9, U.S. Serial Number 60/\_\_\_\_\_, the disclosure of which is incorporated by reference herein as if fully set forth.

Figures 1A through 1F are photomicrographs showing morphological changes induced by NNK in BMRPA1 cells. Figure 1A shows normal appearance of untreated 10 BMRPA1 cells. Figures 1B through 1F show sequential cell passages (1-12) after one 16h treatment of BMRPA1 with NNK. The appearance of spindle cells is first seen in the epithelial monolayer (Fig. 1B). By passage 6 (Fig. 1C) the appearance of round cells is seen on top and within the strands of spindle cells. By passage 7 (Fig. 1D) many foci and beginning of "colony" formation is seen. By passage 9 (Fig. 1E) compact "colonies" 15 (tumors) grew from foci. Fig. 1F shows cells isolated from the core of a large colony and reseeded were spindle shaped and continued to form foci and colonies. Proof of malignant transformation of BMRPA1 cells by NNK was obtained when injection of the above cells subcutaneously (SC) and i.p. into Nu/Nu mice resulted in development of multiple solid tumors in the animals.

EXAMPLE 2Tolerance-induced Targeted  
Antibody Production (TITAP)5 MATERIALS AND METHODS:

Materials: RPMI 1640, DMEM containing 5.5mM glucose (DMEM-G+), penicillin-streptomycin, HEPES buffer, 0.2% trypsin with 2mM EDTA, Bovine serum albumin (BSA), Goat serum, and Trypan blue were from GIBCO (New York). Fetal bovine serum (FBS) was from Atlanta Biologicals (Atlanta, GA). Hypoxanthine (H), Aminopterin (A), and Thymidine (T) for selective HAT and HT media and PEG 1500 were purchased from Boehringer Mannheim (Germany). Diaminobenzidine (DAB) was from BioGenex (Dublin, CA). PBS and Horseradish peroxidase labeled goat anti-Mouse IgG [F(ab')<sub>2</sub> HRP-G<sub>α</sub>M IgG] were obtained from Cappel Laboratories (Cochranville, Pa).

10 Aprotinin, pepstatin, PMSF, sodium deoxycholate, iodoacetamide, paraformaldehyde, Triton X-100, Trizma base, OPD, HRP-G<sub>α</sub>M IgG, and all trace elements for the complete medium were purchased from Sigma (ST. Louis, MO). Ammonium persulfate, Sodium Dodecyl Sulfate (SDS), Dithiothreitol (DTT), urea, CHAPS, low molecular weight markers, and prestained (Kaleidoscope) markers were obtained from BIORAD (Richmond, CA). The enhanced chemiluminescent (ECL) kit was from Amersham (Arlington Heights, IL). Mercaptoethanol (2-ME) and film was from Eastman Kodak (Rochester, N.Y.). Tissue culture flasks (TCF) were from Falcon (Mountain View, CA), tissue culture dishes (TCDs) from Corning (Corning, NY), 24-well TC plates (TCPs) and 96-well TCPs were from Costar (Cambridge, MA). Tissue culture chambers/slides (8 chambers each) were 20 from Miles (Naperville, IL).

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Cells and Culture: All rat pancreatic cell lines were grown in cRPMI containing 10% FBS. The other cell lines were obtained from the American Tissue Culture Collection (ATCC), except for the rat capillary endothelial cells (E49) which were from Dr. M. DelPiano (Max Planck Institute, Dortmund, Germany). White blood cells were from 5 healthy volunteer donors, and human pancreatic tissues (unmatched transplantation tissues) were provided by Dr. Sommers from the Organ Transplantation Division at Downstate Medical Center. Cell viability was assessed by trypan blue exclusion.

Immunosubtractive Hyperimmunization Protocol (ISHIP): A mixture of live ( $10^6$ ) and paraformaldehyde fixed and washed ( $10^6$ ) cells was used for each immunization 10 intraperitoneally (ip). Six female Balb/c mice (age~12 wks) were used: two mice were injected 4X during standard immunizations with BMRPA1 cells. The other four mice were similarly injected 3X with BMRPA1 cells, and 5 h after the last booster injection they were injected ip for the next 5 d with 60  $\mu$ g cyclophosphamide/day/g of body weight. Two of these immunosuppressed mice were re-injected with BMRPA1 cells after the last Cy 15 injection. The other two immunosuppressed mice were injected weekly three more times with transformed BMRPA1.NNK cells, and a week later the mice were hyperimmunized with 5 additional injections in the 7 days preceding fusion (ISHIP mice). Sera were obtained from all mice within a week after the indicated number of immunizations.

Hybridoma and mAb purification: Hybridomas were obtained as previously 20 described (Kohler and Milstein, 1975; Pytowski et al., 1988) by fusion of P3U1 myeloma cells with the splenocytes from the most immunosuppressed ISHIP mouse. Hybridoma cells were cultured in 288 wells of 24-well TCPs. The hybridomas were initially grown in

HAT DMEM-G+ (20% FBS) medium for 10d, followed by growth in HT containing medium for 8d, and then in DMEM-G+ (20% FBS). Hybridoma supernatants were tested 3X by Cell-Enzyme ImmunoAssay (Cell-EIA) starting 3 weeks after fusion for the presence of specific reactivities by Cell-EIA before the selection of specific mAbs for further analysis by immunofluorescence microscopy and immunohistochemistry was made.

EXAMPLE 3Detection of antigenic differences between NNK-transformed and untransformed

BMRPA1 cells: Hybridoma supernatants collected from 288 wells were tested by Cell-Enzyme ImmunoAssay (Cell-EIA) for the presence of IgG antibodies reactive with dried 5 NNK-transformed and untransformed BMRPA1 cells. BMRPA1 and BMRPA1.NNK cells were seeded in TCPs (96-wells) at  $3 \times 10^4$ /well with 0.1 mL cRPMI-10%FBS. The cells were allowed to adhere for 24 h, air dried, and stored under vacuum at RT. The cells were then rehydrated with PBS- 1% BSA, followed by addition of either hybridoma supernatants or two fold serial dilutions of mouse sera to each well for 45 min at room 10 temperature (RT). After washing with PBS-BSA, HRP-G $\alpha$ -M IgG (1:100 in PBS-1% BSA) was added to each well for 45 min at RT. The unbound antibodies were then washed away, and OPD substrate was added for 45 min at RT. The substrate color development was assessed at OD<sub>490nm</sub> with a microplate reader (Bio-Rad 3550). For 15 hybridoma supernatants, an OD<sub>490nm</sub> value greater than 0.20 (5X the negative control OD<sub>490nm</sub> value obtained with unreactive serum) was considered positive. Evaluation on days 18 to 21 after fusion established that 265 (92%) of the 288 wells examined contained one or more growing hybridomas. By Cell-EIA, supernatants from 73 (or 23.5%) of the wells contained antibodies that reacted with transformed BMRPA1.NNK cells. In contrast, only 47 (or 16.3%) supernatants reacted with BMRPA1 cells, indicating that 20 BMRPA1.NNK cells express antigens which are not expressed by the untransformed BMRPA1 cells. Moreover, all 47 hybridoma supernatants reactive with BMRPA1 cells exhibited cross reactivity with transformed BMRPA1.NNK cells.

EXAMPLE 4Immunoreactivity of Selected Hybridoma Supernatants  
with Intact Untransformed and Transformed BMRPA1 cells

As the Cell-EIA testing was performed on dried, broken cells, the antibodies in the supernatants could access and bind both intracellular and plasma membrane Ags. To obtain initial information regarding the cellular location of the recognized Ags, hybridoma supernatants were initially selected for further testing by Indirect Immunofluorescence Assay (IFA) on intact cells because by Cell-EIA these supernatants consistently showed promising strong reactivity either with only BMRPA1.NNK cells (supernatants 3A2; 3C4; 3D4), or with both BMRPA1.NNK and BMRPA1 cells (supernatants 4AB1; 2B5). Supernatants 3C4, 4AB1, and 2B5 stained the cell surface of intact cells in agreement with the Cell-EIA results.

Cells were released by incubation with 0.02 M EDTA in PBS, washed with PBS-1% BSA, and processed live at ice cold temperature for immunofluorescence analysis. The cells were incubated for 1h in suspension with hybridoma supernatants or sera, washed (3X) in PBS-1% BSA, and exposed to FITC-G $\alpha$  M IgG diluted 1:40 in PBS-1% BSA. After 45 min, unbound antibodies were washed away, and the cells were examined by epifluorescence microscopy.

Remarkably, 3C4 stained BMRPA1.NNK (Fig. 2B) and BMRPA1.K-ras<sup>val12</sup> cells (see copending provisional patent application, Serial No. 60/\_\_\_\_\_) in a ring-like pattern, but did not stain the cell surface of untransformed BMRPA1 cells (Fig. 2C), suggesting the presence of the 3C4-Ag on the surface membrane of only transformed cells.

### EXAMPLE 5

#### Immunoperoxidase Staining of Permeabilized Cells and Tissue Sections.

*Preparation of cells and tissues:* Transformed and untransformed BMRPA1 cells were seeded at 1x 10<sup>4</sup> cells/0.3 mL cRPMI/chamber in Tissue Culture Chambers. Two days later, the cells were fixed in 4% paraformaldehyde in PBS overnight at 4°C. The cells were then washed twice with PBS-1% BSA and used for immunocytochemical staining.

Pancreatic tissue for immunohistochemical staining was prepared from adult rats perfused with 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2. The fixed pancreas was removed from the fixed rat and stored overnight in 4% buffered paraformaldehyde at 4 °C.

The pancreas was then washed and placed in 30% sucrose overnight. Frozen tissue sections (10 µm) were made with a Jung cryostat (Leica), placed on gelatin-coated glass slides, stored at -20 °C. The cell lines or tissue sections were then post-fixed for 1 min in 4% buffered paraformaldehyde, washed in Tris buffer (TrisB) (0.1M, pH 7.6), and placed in Triton X-100 (0.25% in TrisB) for 15 min at RT. Immunohistochemistry was then performed as previously described (Guz et al., 1995).

IF staining with mAb3C4 of live rodent and human PaCa cells localized the 3C4-Ag to the plasma membrane of the intact cells (Figures 6A through 6J). The 3C4 staining detected by IFA and FACS (Example 6) was totally abolished when trypsin/EDTA instead of only EDTA was used to release the cells, indicating that the 3C4 Ag is a trypsin-sensitive protein found on the outer membrane of transformed BMRPA1 cells.

EXAMPLE 6

**Fluorescence Activated Cell Sorting Analysis (FACS) of  
Transformed and Untransformed Rodent and Human Pancreatic Carcinoma Cells**

5       Live cells were placed on ice and reacted sequentially with mAb3C4 and  
Fluorescein Isothiocyanate (FITC-) labeled rabbit- $\alpha$ M IgG (FITC-R- $\alpha$ M IgG), fixed  
overnight in 2% buffered paraformaldehyde, washed and analyzed on a BD FACS IV  
analyzer.

10      FACS analysis of stained BMRPA1.TUC3 cells provided a semi quantitative  
assessment of the presence of the antigen on the surface of the cells and confirmed  
fluorescence on >99% of the cells, indicating that >99% of the cells in each of the PaCa  
cell population expressed the 3C4-Ag. These results are shown in the scattergrams and  
fluorescence intensity graphs of Figure 7.

EXAMPLE 7Purification of mAb3C4

5 Mice were injected with 3C4 hybridoma cells ( $10^7$ /mouse). Ascites were collected and mAb3C4 IgG1 was purified from the ascites using G-protein affinity beads. Protein G beads were incubated under constant rotation overnight at 4°C with ascites extracted from mice injected intraperitoneally (i.p.) with mAb3C4-producing hybridoma cells. The protein G beads were then centrifuged, the supernatant was removed, and the beads  
10 washed sequentially with Buffer A (10 mM Tris, 2 mM EDTA, 100 mM NaCl, pH 7.5), Buffer B (10 mM Tris HCl, 200 mM NaCl, 2 mM of EDTA, 0.2% Triton X-100, 0.25 mM PMSF pH 7.5), and Buffer C (10 mM Tris HCl, 0.25 mM PMSF pH 7.5) to remove non-specifically adsorbed proteins. Bound mAb3C4 was eluted from the beads with two bead volumes of elution buffer (0.1 M Glycine pH 2.7) followed each time by neutralization of  
15 the eluate with 1M Tris-HCl, pH 9.0 after its separation from the beads by brief centrifugation.

The purification of the mAb3C4 IgG was confirmed by SDS-PAGE and Immunoblotting (IB).

SDS PAGE and Immunoblotting (IB) of mAb3C4:

20 The mAb3C4 eluted and separated from the protein G-beads column were subjected to SDS PAGE under reducing and non-reducing conditions and immunoblotting (IB). mAb3C4 samples as well as other samples described below, were mixed with equal volumes of non-reducing sample buffer (125mM Tris-HCl, 2% SDS, 0.1% bromophenol

blue, 20% v/v glycerol, pH 6.8) and reducing sample buffer (125mM Tris-HCl, 2% (v/v) 2-mercaptoethanol, 2% SDS, 0.1% bromophenol blue, 20% v/v glycerol, pH 6.8) The proteins from each sample (20 µg/well) were separated by SDS-PAGE as previously described (Laemmli, 1970), and electrotransferred onto nitrocellulose membrane. Gel

5 lanes were loaded as follows:

	<u>Lane</u>	<u>Sample</u>
	1	Hybridoma injected mouse ascites
	2	Low pH buffer elution of proteins from protein-G beads incubated with ascites
10	3	Proteins of Lane 2 after Reduction
	1B	IB of Lane 1
	2B	IB of Lane 2

15 After the membrane was incubated with 5% (w/v) dry milk in TBS-T for 1h, the HRP-G α M IgG antibody was used as suggested by the manufacturer (ECL kit, Amersham). The presence of the mAb3C4 protein by ECL in each of the samples tested was detected by exposure to X-OMAT film (Kodak).

Figure 3, lanes 1-3, is a photograph of a Coomassie blue stained SDS gel run with 20 G-protein affinity purified mAb3C4 from ascites. Lane 1 indicates significant quantities of mAb3C4 were released into the ascites as seen by the bulge around ~150-160 kD region. Lane 2: low pH elution where IgG was quantitatively released from the bead. Lane 3 shows the ~160 kD protein (IgG) of lane 2 reduced. The disappearance of the ~160 kD protein and the appearance of ~55 kD heavy and ~28 kD light chains typically of IgG are evidence that the extracted 160 kD protein is in fact IgG. Lanes 1B and 2B depict 25 immunoblots and autoradiograms (chemiluminescentograms) of the IgG in lanes 1 and 2

using HRP-SaM IgG and ECL reaction kit, confirming the ~160 kD protein to be IgG. This purification resulted in extraction of about 2/3 of the antibodies present in the ascites and succeeded in removal of >98% of contaminants. ELISA analysis for isotype specificity identified mAb3C4 to belong to the IgG1 subclass of mouse IgG with kappa 5 light chain.

EXAMPLE 8Identification of the 3C4 Antigen (PaCa-Ag1)

SDS PAGE of cell lysate proteins from rodent and human pancreatic carcinoma  
5 cells followed by IB with mAb3C4 was used to identify the protein nature and the molecular weight (MW) of 3C4-Ag. Cells were grown to confluence in 25cm<sup>2</sup> TCDs, washed with ice-cold PBS, and incubated on ice with 0.5 mL RIPA lysing buffer (pH 8) consisting of 50mM Tris-HCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 5mM EDTA, 1μg/mL pepstatin, 2ug/mL aprotinin, 1mM PMSF, and 5mM iodoacetamide. After 10 30 min, the remaining cell debris was scraped into the lysing solution, and the cell lysate was centrifuged at 11,500 x g for 15 min to remove insoluble debris. The protein concentration of each lysate was determined by the Bradford's assay (BioRad). The cell extracts were mixed with equal volumes of non-reducing sample buffer (125mM Tris-HCl, 2% SDS, 0.1% bromophenol blue, 20% v/v glycerol, pH 6.8) or reducing buffer (125 mM 15 Tris-HCl, 2%(v/v) 2-mercaptoethanol, 2% SDS, 0.1% bromophenol blue, 20% v/v glycerol, pH 6.8). The proteins from each sample (20.μg/well) were separated by SDS-PAGE as previously described (Laemmli, 1970), and electrotransferred onto nitrocellulose membrane. Gel lanes were loaded as follows:

Lane    Sample

- 20    1 =    BMRPA1.NNK + mAb3C4 + HRP-SaM IgG;  
      2 =    BMRPA1 + mAb3C4 + HRP-SaM IgG;  
      3 =    BMRPA1.NNK + HRP-SaM IgG;  
      4 =    MIA PaCa + HRP-SaM IgG;

5 = Reduced MIA PaCa + HRP-SaM IgG;

6 = Reduced MIA PaCa + mAb3C4 + HRP-SaM IgG;

7 = MIA PaCa + mAb3C4 + HRP-SaM IgG

After the membrane was incubated with 5% (w/v) dry milk in TBS-T for 1h,  
5 mAb3C4 (1:200) and the HRP-G  $\alpha$  M IgG antibody were used as suggested by the  
manufacturer (ECL kit, Amersham). The presence of the protein of interest by ECL in  
each of the samples tested was detected by exposure to X-OMAT film (Kodak).

As shown in the immunoblot depicted in Figure 4, the mAb3C4 clearly identified  
the 3C4-Ag to be a ~43 kD protein present in NNK transformed cells and Human PaCa  
10 cell line MIA PaCa-2. The fact that reduction does not change the migration pattern of  
3C4-Ag indicates that the antigen does not contain subunits.

**EXAMPLE 9****2D Isoelectric focusing/SDS-Duracryl Gel Electrophoretic Polypeptide Separation**

BMRPA1.NNK cells were lysed *in situ* in the presence of protease inhibitors, their nuclei removed by centrifugation, and the protein concentration of the cell lysate established by Bradford's assay (BioRad). Cell protein (0.4mg) was transferred into 5 isoelectric focusing sample buffer made with urea-/NP-40-solution (8.15ml) and 2-mercaptopethanol (0.2ml) in dH<sub>2</sub>O (1.65ml) [urea-/NP-40 stock solution: 24g urea dissolved in 18ml dH<sub>2</sub>O containing 0.84ml NP-40 (Nonidet)]. The lysate in sample buffer was then placed on top of IEF capillary tube gel consisting of acrylamide/bis-acrylamide 10 (0.5ml), urea-/NP-40 solution (3.76ml), biolyte mixture (0.25ml) ammonium sulfate (0.015ml of 10% w/v solution) and TEMED (0.004ml). Acrylamide/bis-acrylamide mixture was prepared with 9 g acrylamide and 0.54g bis-acrylamide dissolved in 30ml dH<sub>2</sub>O. Biolyte (ampholine) mixture was made by combining Biolytes covering ranges from 3-10 (0.4ml) and 5-7 (0.1ml). Proteins were separated on the IEF gel for 2h 15 at 200V followed by 5h at 500V and 16h at 800V. The second dimension defining the molecular weights of the separated proteins was run in a 12% SDS-PAGE gel (BioRad) at 20mA/gel. Several IF and SDS-PAGE gels were run in parallel under identical conditions and processed for silver staining (Genomic Solutions Inc.) (Figure 11) and electrophoretic transfer to PVDF membrane (Schleicher and Scholl) for immunoblotting with mAb3C4 20 (Figure 12) and to Immobilon membrane for the isolation of the 3C4-Ag spot for protein sequencing. Prestained molecular markers were used to verify appropriate transfer of the proteins from the IF gel to the membranes. The silver staining in Figure 11 shows

the presence of a large number of individual proteins in the cell lysate and their appropriate separation according to their PI values, within the IF gel. The immunoblot pictured in Figure 12 was developed using the ECL-chemiluminescence procedure on X-ray film. The chemiluminescentogram of the mAb3C4 blot shows only a single spot of luminescence 5 (arrow head) which identifies the 3C4-Ag as a ~ 43 kD polypeptide with a pI of 4.6-4.8.

The separated polypeptides were either rapidly transferred onto a PVDF (Schleicher and Scholl) membrane under semi-dry conditions for one hour at 1.25 mA/cm<sup>2</sup> (484 mA), or, stained with a silver kit according to the manufacturer's instructions (Genomics Solutions, MA). The PVDF membrane was used for 3D4-Ag detection by 10 Western blot analysis, and was later stained with either Rev Pro (Genomic Solutions, MA), or Amido Black. The pH gradient in the first dimension was determined from 1.0 cm sections as previously described (O'Farrell, 1975). The silver staining of the 2D separated polypeptides was recorded by computer scanning of the gel.

**EXAMPLE 10****Expression of the 3C4 Ag is Highly Restricted to Pancreatic Cancer Cells and Absent from Normal Tissues**

- 5 To examine the distribution of the 3C4-Ag within normal rat, human tissues and transformed human tissues, an immunoblot of tissue extracts using mAb3C4 was performed. Reduced proteins from tissue extracts from various tissues (thyroid, ovary, brain, heart, lung, liver, testes, see Fig. 9A) as well as human acinar pancreatic cells, white blood cells, and ductal pancreatic cells (see Fig. 9B) were separated on 12% SDS PAGE,
- 10 electrophoretically transferred to nitrocellulose and processed with and without mAb3C4 followed by ECL chemiluminescence amplification. MIA-PaCa and mouse IgG served as controls. The extracts (0.05 mg/lane) of reduced proteins were separated on 12% SDS PAGE, electrophoretically transferred to nitrocellulose and processed with and without mAb3C4 followed by ECL chemiluminescence amplification (Amersham Pharmacia).
- 15 Human pancreatic acinar (PA) and ductal tissues (PD), were loaded 10X and 4X respectively, in order to rule out the presence of even minute quantities of the expression of the Ag. MIA PaCa-2 cell lysate and IgG were used as controls. Results as set forth in Figure 9, indicate that the 3C4 Ag is absent from normal tissues but present in pancreatic cancer cells.
- 20 An immunoblot of various human cancerous tissue (glioblastoma, lung cancer, epidermal cancer, colorectal ACA, breast cancer ACA, epidermal ACA, renal ACA, MIA PaCa) using mAb3C4 was then performed, with the results set forth in FIGURE 10. The results demonstrate a highly selective reactivity of mAb3C4 for an antigen of 43 kD, the

3C4-Ag strongly expressed in human PaCa, MIA PaCa-2 cells. The specificity of the reactivity is further demonstrated by an absence of any protein band in all tissue samples when mAb3C4 was omitted during the IB or replaced by non-specific IgG. There appears to be present small quantities of the 3C4-Ag in renal, prostate and possibly colon carcinoma, although the amount appears insignificant compared to the amount expressed by PaCa cells of which only .02 mg of protein were separated in the lanes shown. Taken together, the results obtained by IB and IC strongly support the specificity of mAb3C4 for an antigen, 3C4-Ag, that is preferentially expressed in rat and human PaCa cells.

**EXAMPLE 11****Demonstration of Complement-mediated Cytotoxicity of mAb3C4 to PaCa cells**

5       The Cytotoxicity of mAb3C4 was determined as follows: Human MIA PaCa-2 cells were incubated with mAb3C4 at 4° C followed by incubation in fresh rabbit serum as a source of complement (C) at 37 ° C. The results, set forth in FIGURE 8, show that with increasing concentration of C at a constant concentration of mAb3C4, an increasing number of cell lysis was obtained. In contrast, even at the highest concentration, HI-C  
10      (HI-C = Heat inactivated rabbit serum, 56 °C, 45 mins) was equally ineffective in demonstrating cytotoxicity towards MIA PaCa-2 cells as was C in the absence of mAb3C4. Similar results were obtained for BMRPA1.NNK and BMRPA1.Tuc3 cells used in this assay. All dilutions were made in PBS containing Ca<sup>++</sup> and Mg<sup>++</sup>.

15

**EXAMPLE 12****Effect of mAb3C4 on Tumor Growth *in vivo***

Nu/Nu mice (n=10) were xenotransplanted with BMRPA1.TUC3 cells ( $5 \times 10^6$  cells/mouse) subcutaneously. Tumors were allowed to develop and grow until they  
5 reached diameters of from 10 to 14 mm. At this time, 3C4 hybridoma cells secreting mAb3C4 were injected intraperitoneally (ip) at  $10^6$  cells per mouse. Subsequently, at 2 day intervals, tumor development was observed and the diameter of tumors measured. Within 4 days, tumor growth was arrested and within 16 days, tumor size regressed to values of between 4-6 mm in diameter, i.e., significantly below the size measured initially at the  
10 time of 3C4 hybridoma IP injection. Significance value of tumor regression is < 0.00066 as determined using mixed model analysis.

WHAT IS CLAIMED IS:

1. A pancreatic carcinoma-specific antigen 3C4-Ag in substantially purified form characterized by:
  - a molecular weight of about 43 kDa as determined by SDS-PAGE;
  - 5 a pI on isoelectrofocusing of about 4.5 to about 5.0; and; being primarily localized on the surface of rat and human pancreatic cancer cells but not detected in normal, non-proliferating cells.
2. An immunologically active fragment of the pancreatic carcinoma-specific antigen 3C4-Ag of claim 1.
- 10 3. An antibody or binding portion thereof, having binding specificity to pancreatic carcinoma specific antigen 3C4-Ag, wherein said antigen is characterized by:
  - a molecular weight of about 43 kDa as determined by SDS-PAGE;
  - a pI on isoelectrofocusing of about 4.5 to about 5.0; and; being primarily localized on the surface of rat and human pancreatic cancer cells
- 15 but not detected in normal, non-proliferating cells.
4. The antibody of Claim 3 which is a polyclonal antibody.
5. The antibody of claim 3 which is a monoclonal antibody.
6. A murine hybridoma cell line which produces a monoclonal antibody specifically immunoreactive with the 3C4-Ag antigen of Claim 1.
- 20 7. A murine hybridoma cell line which produces the monoclonal antibody of Claim 5.

8. A monoclonal antibody, mAb34C, secreted by the hybridoma cell line of

Claim 6.

9. The monoclonal antibody mAb3C4 of claim 4 or 8 in a humanized form.

10. An antibody according to any one of claims 3-5, or 8 wherein the antibody  
5 is labeled with a fluorophore, chemilophore, chemiluminecer, photosensitizer, suspended  
particles, radioisotope or enzyme.

11. An antibody according to claim 9 wherein the antibody is labeled with a  
fluorophore, chemilophore, chemiluminecer, photosensitizer, suspended particles,  
radioisotope or enzyme.

10 12. An antibody according to any one of claims 3-5, or 8 wherein the antibody  
is conjugated or linked to a therapeutic drug or toxin.

13. An antibody according to claim 9 wherein the antibody is conjugated or  
linked to a therapeutic drug or toxin.

14. A method of detecting pancreatic cancer in an animal subject, said method  
15 comprising the steps of:

(a) contacting a cell, tissue or fluid sample from the subject with at least one of an  
antibody or binding portion thereof which specifically binds to 3C4-Ag or an  
immunologically active fragment thereof; the monoclonal antibody mAb34C; or  
an antibody which binds the epitope bound by the monoclonal antibody mAb34C;  
20 under conditions permitting said antibody to specifically bind an antigen in the  
sample to form an antibody-antigen complex;

(b) detecting antibody-antigen complex in the sample; and

(c) correlating the detection of elevated levels of antibody-antigen complex in the sample with the presence of pancreatic cancer.

15. A diagnostic kit suitable for detecting 3C4-Ag in a cell, tissue, or fluid sample from a patient, said kit comprising:

5 (a) an antibody or binding portion thereof which specifically binds 3C4-Ag or an immunologically active fragment thereof,

(b) a conjugate of a specific binding partner for the antibody or binding portion thereof, and

(c) a label for detecting the bound antibody.

10 16. A method of treating pancreatic cancer in a patient suffering therefrom which comprises administering to the patient an effective amount of an antibody or binding portion thereof which specifically binds to 3C4-Ag or an immunologically active fragment thereof, wherein said antibody or binding portion thereof is conjugated or linked to a therapeutic drug or toxin.

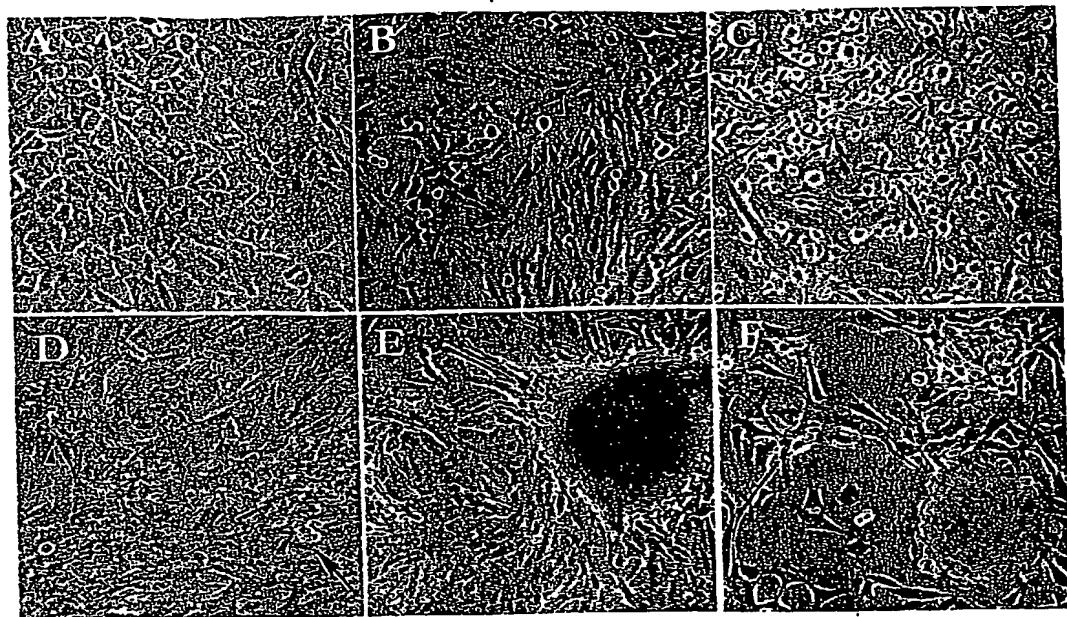
15 17. The method of claim 16 wherein said antibody is mAb3C4.

18. A pharmaceutical composition comprising an antibody or binding portion thereof which specifically binds to 3C4-Ag, admixed with a pharmaceutically acceptable carrier.

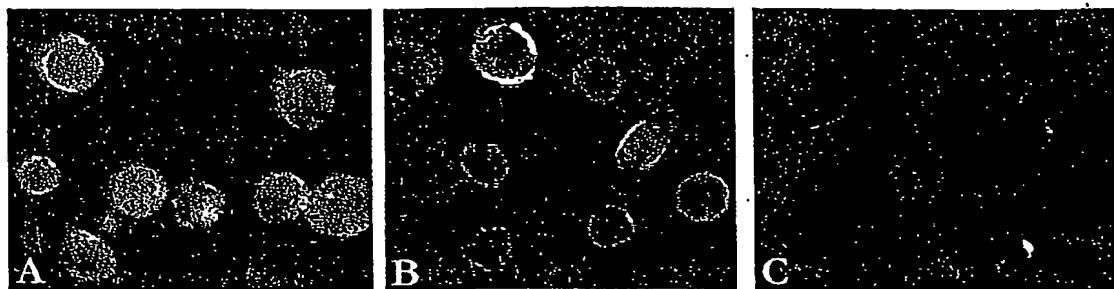
20 19. The pharmaceutical composition of claim 18 wherein the antibody or binding portion thereof which specifically binds to 3C4-Ag is conjugated or linked to a therapeutic drug or toxin.

**ABSTRACT**

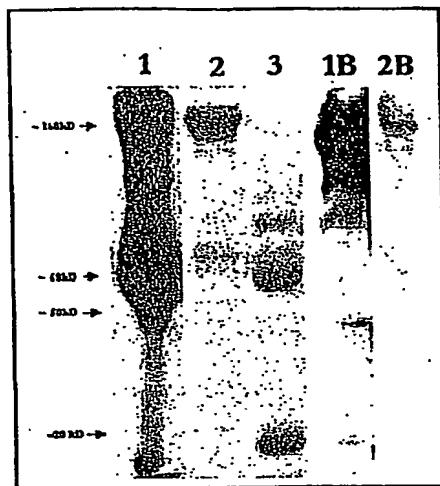
The present invention is directed to an antigen found on the surface of rat and human pancreatic cancer cells, antibodies of high specificity and selectivity to this antigen, and methods for both the diagnosis and treatment of pancreatic cancer.



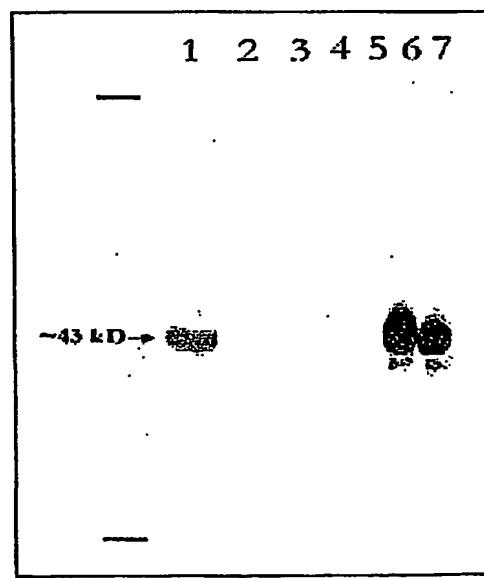
**FIGURES 1A-1F**



**FIGURES 2A-2C**



**FIGURE 3**



**FIGURE 4**

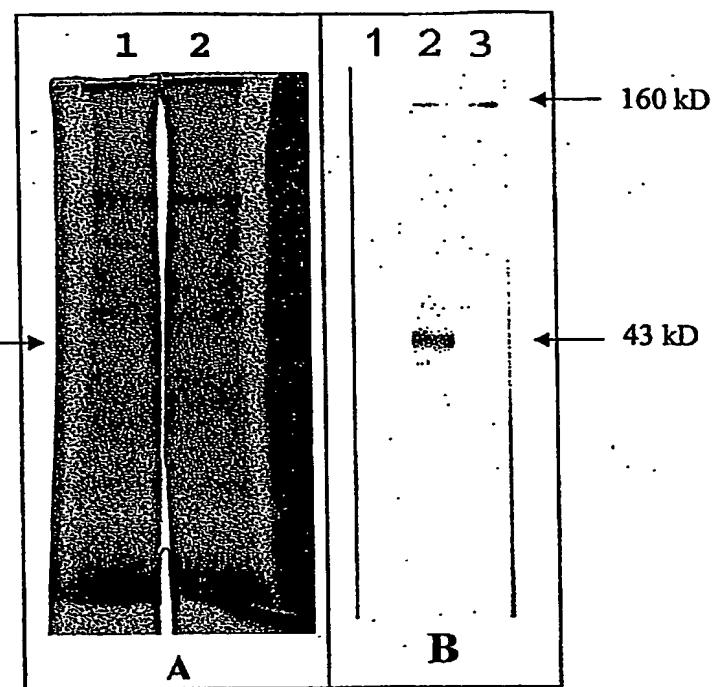
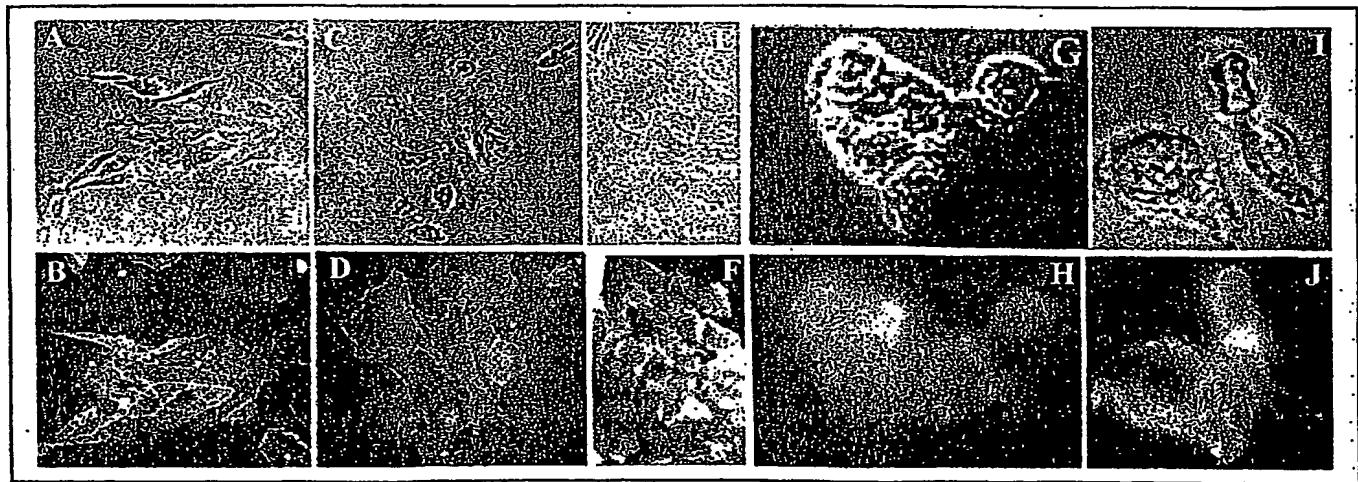
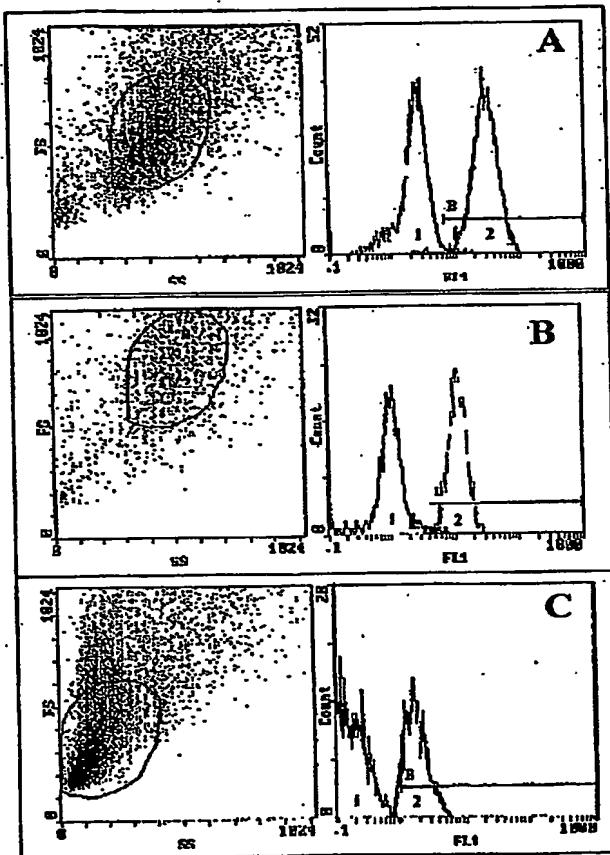


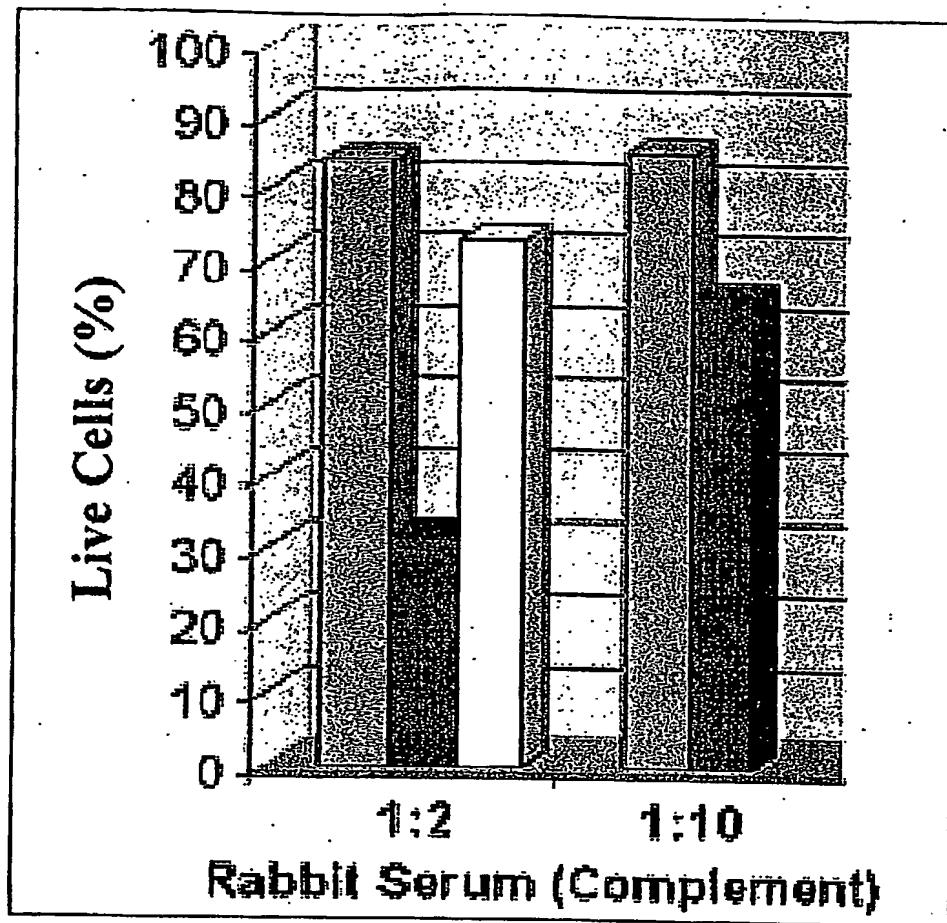
FIGURE 5



FIGURES 6A-6J

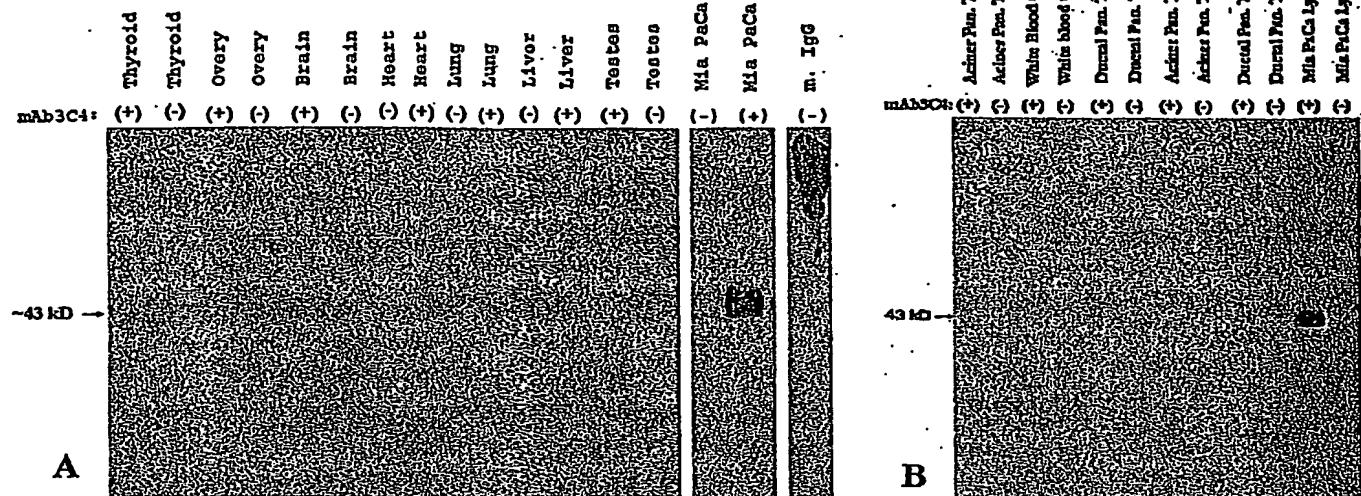


**FIGURES 7A-7C**

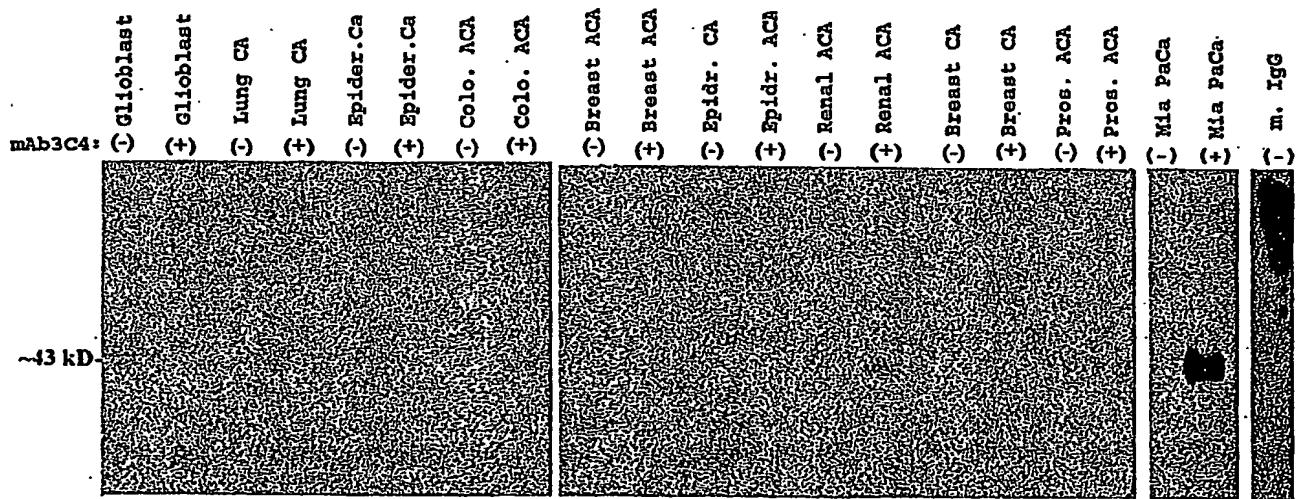


**FIGURE 8**

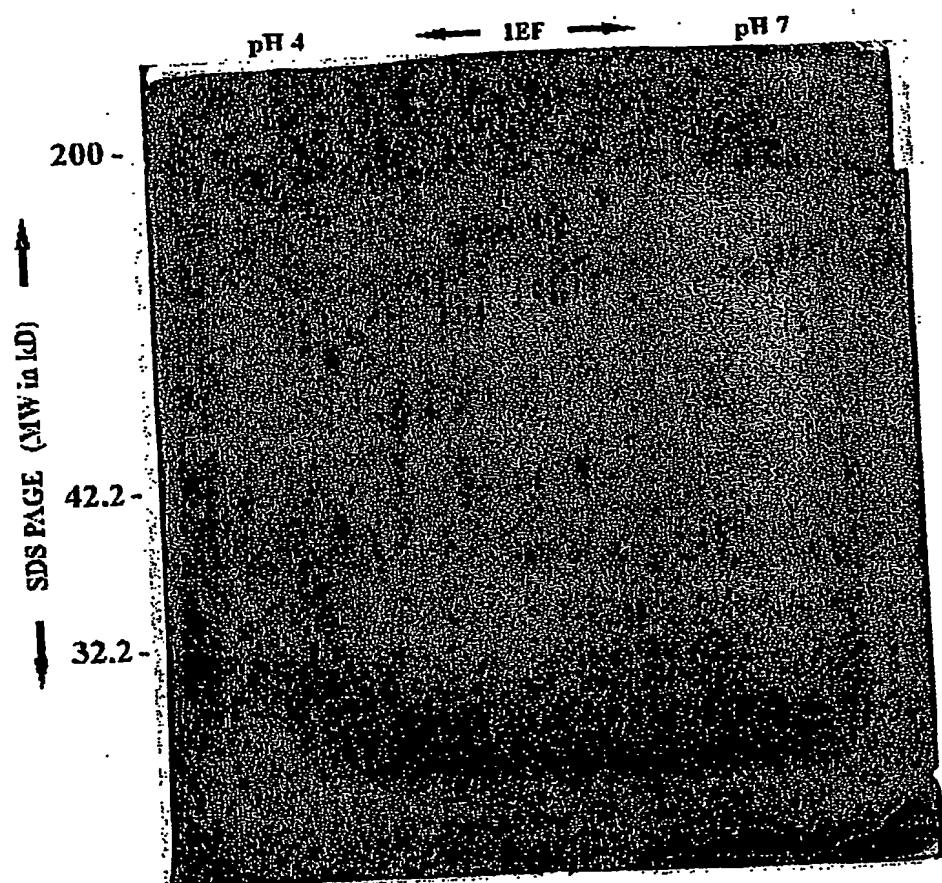
65-10699 . B11703



## **FIGURE 9A-9B**

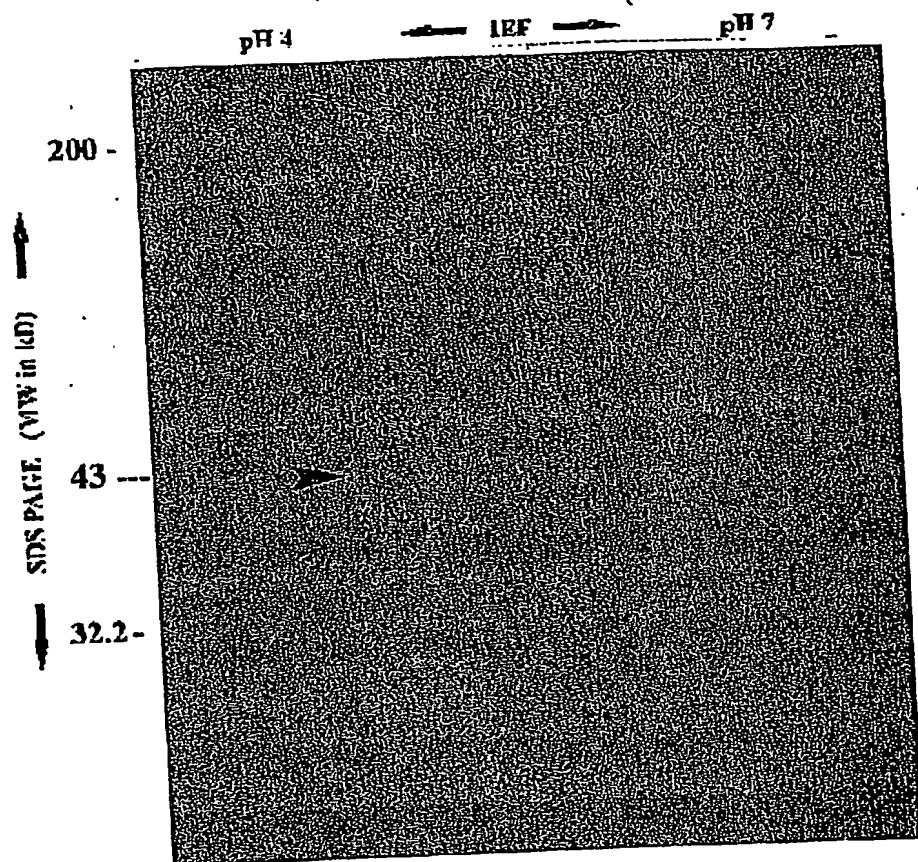


## FIGURE 10.

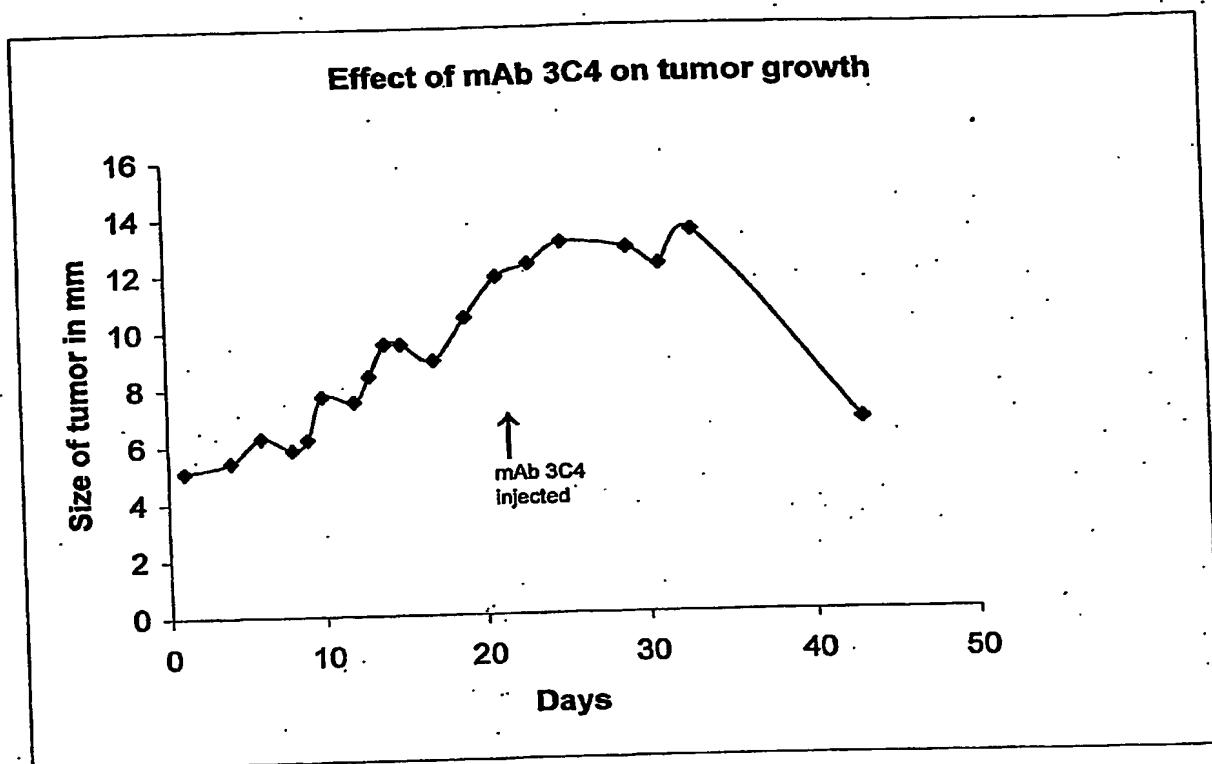


**FIGURE 11**

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**FIGURE 12**



**FIGURE 13**

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